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Award Number: DAMD17-03-1-0723

TITLE: MIC-1, A Potential Inhibitor of Breast Tumor Progression

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REPORT DATE: October 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2004	Annual (2 Sep 03 - 1 Sep 04)

4. TITLE AND SUBTITLE
MIC-1, A Potential Inhibitor of Breast Tumor Progression

5. FUNDING NUMBERS
DAMD17-03-1-0723

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

MIC-1 is a member of the transforming growth factor-beta family of growth and differentiation factors. MIC-1 is expressed in breast tissue and has numerous specific effects including inhibiting both breast development and breast duct proliferation. We hypothesize, therefore, that MIC-1 may play a role in inhibiting breast cancer progression. In order to study this question we have generated genetically modified mi in which murine MIC-1 expression is abolished (null mice). We are in the process of breeding these MIC-1 null mice with mouse lines that are predisposed to the development breast cancer and this will be examined in the upcoming year as a no-cost extension of this grant. We will analyze the effects of the absence of MIC-1 on the rate of breast malignancy development in these animals comparing it to those that have intact MIC-1 production. If our hypothesis is correct, MIC-1 null mice will exhibit increased incide and/or severity of breast cancer. As such, these studies may identify an important anti tumor pathway in breast cancer, potentially providing novel strategies and targets for chemical therapeutics or diagnosis.

14. SUBJECT TERMS
MIC-1, Gdf-15, Breast, Cancer

15. NUMBER OF PAGES
6

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Introduction:

MIC-1 is a member of the transforming growth factor-beta family of growth and differentiation factors that may play a role in inhibiting breast cancer progression. This grant seeks to breed MIC-1 null mice with mouse lines that are predisposed to the development of breast cancer and analyze the rate of breast malignancy development in these animals compared to those that have intact MIC-1 production. Of note, due to breeding problems and a death of our animals in our animal care facility the grant is undergoing a one year no cost extension to complete the project.

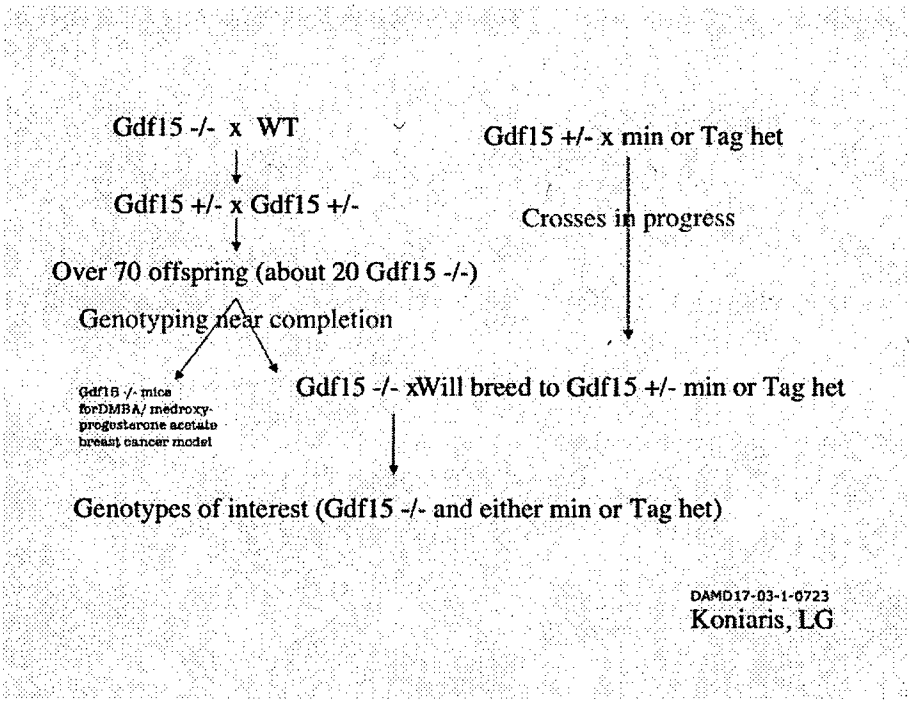
Body:

Female C57BL/6MIC-1 -/- animals are in the process of being bred to two distinct commercially available (Jackson Labs, Bar Harbor, ME) genetically modified mice lines that demonstrate an increased incidence of breast malignancies. The first mouse line over-expresses SV40 large T antigen on a breast -specific promoter (TgN(C3-1-TAg). The second mouse line has a modified APC gene (adenomatous polyposis coli), i.e. the Min (multiple intestinal neoplasia) mouse. It is unusual for either of the breast tumor-prone mouse lines to survive beyond 6 months of age due to the development of malignancies. Both commercially available mouse lines are in the process of being bred into a MIC-1 -/-background. F1 animals will be screened and bred again into the MIC-1 -/- mice to obtain MIC-1/GDF-15 null mice that also express large T antigen (TgN(C3-1-TAg), gdf15-/-) or also possess a modified APC gene (Min+/-, gdf15-/-). Age and sex-matched (TgN(C3-1-TAg), gdf15-/-), (Min+/-, gdf15-/-),(TgN(C3-1-Tag), gdf15+/+), and (Min +/-, gdf15+/+) mice will be obtained. Ten female animals of each category will be sacrificed and subjected to necropsy atl, 3 or 5 months of age. A determination of whether the number and/or frequency of breast and other tumor formation occurs in animals without MIC-1 will be determined.

At this juncture, we currently have 20 male GDF15 -/+ and 12 female GDF15 -/+ mice that are being used for breeding. One cage has a homozygous male and hetero female with the APCmin/+ genotype. Of the 16 breeding cages 5 are currently with pups. We have 50 weaned offspring who are products of a two hetero parents, 21 males and 29 females respectively. Exact genotypes of the offspring are pending

southern blotting prior to their use as breeding pairs. Thus, we are well on the way to generating the mice of interest following the unfortunate animal care issues.

Update: 3/22/05: Please see summary table. Note: at time of award we had 50 gdf15-/- animals for the proposed project. In quarantine at the University of Miami, however, these animals died. We thus were required to regenerate sufficient numbers of the required genotypes for the proposed experiments.



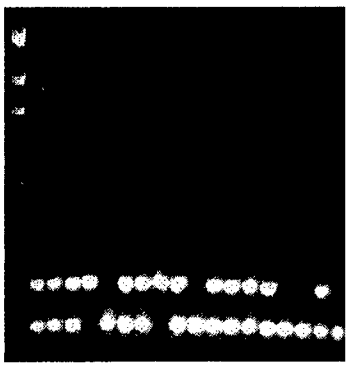
We now have over 70 mice that are the product of the heterozygous expansion of the gdf15+/- matings. We currently are genotyping these animals using a PCR protocol we have developed to the c-terminal domain of the gdf15 gene and to the G418 resistance insert. Following this round of matings as well as gdf15 heterozygous matings to each of the above tumor-prone lines we should be close to generating the genotypes for the proposed study. Of note, we have encountered some difficulty in the viability of min heterozygote mice and will plan to also modify our approach and also examine the effect of gdf15 -/- on the DMBA/ medroxy-progesterone acetate breast cancer model. This additional objective will allow examination of gdf15 in a tumor progression model in shorter order.
Additional note: the min and T-antigen lines were chosen for the proposed experiment as they get a number of malignancies in addition to breast cancer and it is our

intention to fully analyze these animals whether or not we are able to complete this in the time table of the DOD grant. We have, for example, received additional unrestricted funding from regional cancer societies which will also help complete this challenging but important study.

Key research accomplishments:

Marked mouse colony expansion to generate the mice of interest

Update: 3/22/05
We have developed a pcr-based genotyping model for gdf15 animals. Below is a photo of a 2% agarose gel following PCR amplification of the c-terminal gdf15 region and the G418 cassete. Lanes with larger product only represent gdf15-/-.



Reportable outcomes:

In progress

Conclusions:

In progress

References:

None